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VOLUME XLVIII NUMBER 4

BOTANICAL GAZETTE

OCTOBER 1909

THE EMBRYO SAC OF HABENARIA[1]

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(WITH TWELVE FIGURES)

The present investigation was made on *Habenaria ciliaris* (Michx.) R. Br. and *H. integra* (Nutt.) Spreng. The development of the two species is very much alike and the same description will apply to both. The material was fixed in medium chromacetic and the sections were cut 10 μ thick and stained with Flemming's triple or Haidenhain's iron alum hematoxylin.

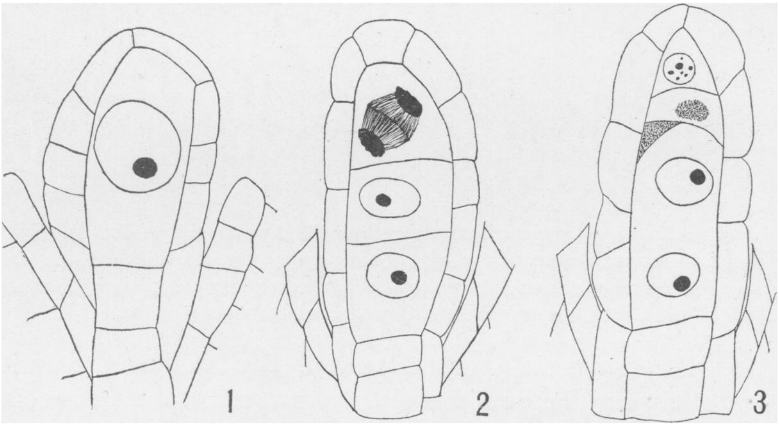

FIG. 1. Megaspore mother cell in apex of nucellus.—FIG. 2. Megaspores.—FIG. 3. First division of functional megaspore and three degenerating megaspores.

The ovule of Habenaria is anatropous and has two integuments. The archesporium differentiates in the apex of the nucellus as a single hypodermal cell (*fig. 1*), which is the terminal cell of a row surrounded only by the epidermal layer. The archesporial cell without dividing functions as a megaspore mother cell, which divides to two daughter cells, which in turn divide to form four megaspores. The last division may be simultaneous in both daughter cells, but usually it is delayed in the one nearest the micropyle (*fig. 2*).

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[1] Contribution from the Botanical Laboratory of the Johns Hopkins University, No. 10.

Soon after the formation of the megaspores the chalazal one begins to enlarge at the expense of the other three, which soon degenerate. Before this degeneration has proceeded far the nucleus of the functioning megaspore divides (*fig. 3*) and the two daughter nuclei remain in the polar positions in the enlarging sac. Each of these two nuclei by two successive divisions gives rise to four, so that the mature sac contains eight nuclei (*fig. 5*). As the sac continues to enlarge, the nucellar cells which surrounded the four megaspores degenerate, so that at about the four-nucleate stage the sac comes to lie against the inner integument. At the last division of the embryo sac nuclei the two spindles in each end are arranged approximately at right angles to one another (*fig. 4*). As is usual, the transverse spindle in the micropylar end gives rise to two synergids, while the longitudinal one forms the egg and the micropylar polar nucleus. In the chalazal end the longitudinal spindle gives rise to the antipodal polar nucleus and one

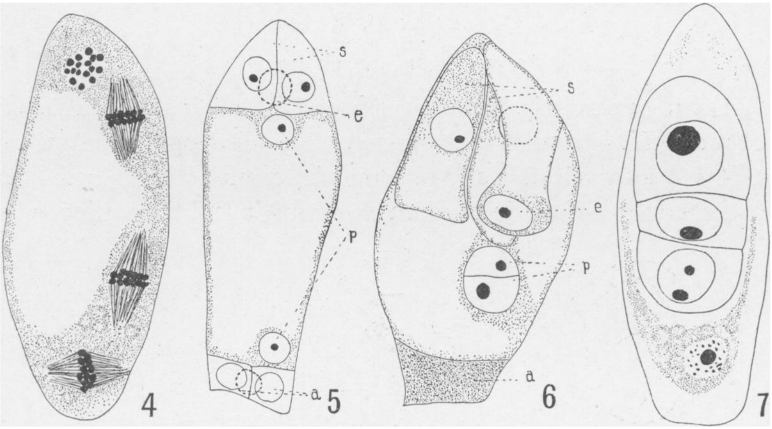
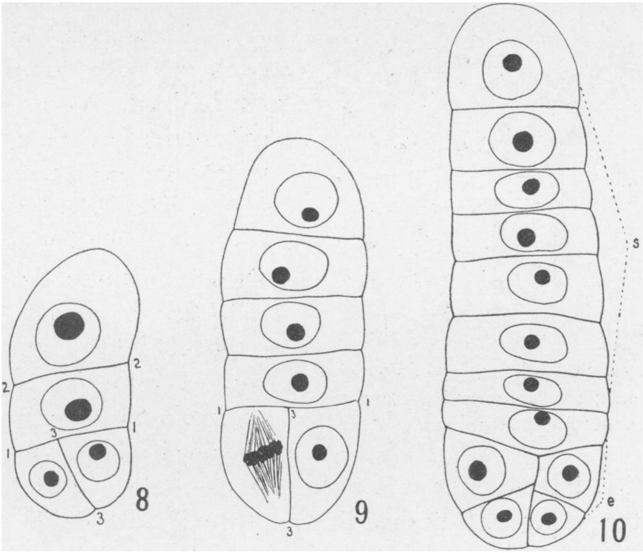


FIG. 4. Nuclei of four-nucleate embryo sac dividing to eight.—FIG. 5. Eight-nucleate embryo sac: *s*, synergids; *e*, egg; *a*, antipodals; *p*, polar nuclei.—FIG. 6. Embryo sac with fusing polar nuclei; *p*, polar nuclei; *s*, synergids; *e*, egg; *a*, degenerating antipodals.—FIG. 7. Young embryo and degenerating endosperm nucleus.

antipodal, while the other spindle forms two antipodals. Walls cutting off the egg, synergids, and antipodals are formed on fibers connecting the nuclei. Owing to their position nearer the center of the sac, the polar nuclei are left free in the cytoplasm (*fig. 5*), as has been pointed out by STRASBURGER ('05) for *Drimys Winteri.* After their formation, the egg and synergids enlarge considerably, while the antipodals soon degenerate (*fig. 6*). When the egg and synergids have about reached their mature size, the polar nuclei fuse (*fig. 6*). No cases were observed in which the polar nuclei were in contact without fusing, as have been described for other forms by STRASBURGER ('00) and NAWASCHIN ('00). The pollen tube comes through the micropyle and discharges the two male nuclei into the embryo sac. One of these fuses with the egg, while the other fuses with the product of the fusion of the polar nuclei to form the primary endosperm nucleus. This enlarges somewhat, but without dividing begins to degenerate at about the time of the first division of the fertilized egg (*fig. 7*).



FIGS. 8–10. Older embryos: *s*, suspensor; *e*, embryo.

The first division of the fertilized egg is transverse to the longitudinal axis of the embryo sac. Of the two resulting cells the chalazal one forms most of the embryo, while the micropylar one gives rise to the suspensor and a small part of the young embryo. The second division (*fig. 8, wall 2*) is in the micropylar cell and is also transverse; the third division (*fig. 8, wall 3*) is in the chalazal cell and is longitudinal. Divisions continue in the descendants of the micropylar cell until a row of about eight cells is formed (*fig. 10*). The seven of these which are nearest the micropyle make up the suspensor, while later on the other one divides to form part of the embryo (*fig. 11*). The first division in this cell may be either transverse or longitudinal. When the row of cells derived from the primary micropylar cell has become four or more cells long, transverse segmentation begins in the descendants of the chalazal cell (*fig. 9*), so that this becomes cut into quadrants (*fig. 10*). Up to this stage the embryo and suspensor have remained within the embryo sac cavity, but now the cells of the suspensor elongate and the free end of the suspensor is pushed through the micropyle out beyond the integuments (cf. shape of cells in *figs. 10* and *11*). The cells of the embryo undergo further division and form a globular mass; *fig. 12* represents a young embryo in which *walls 1* and *3* correspond to *1* and *3* of *figs. 8–11*.

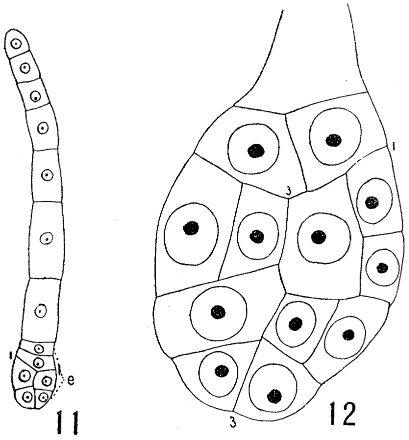


FIG. 11. Still older embryo drawn on a smaller scale; the suspensor has elongated. —FIG. 12. Many-celled embryo.

Discussion

MEGASPORES

In Habenaria the megaspore mother cell divides to two daughter cells and each of these divides to two megaspores. The division in the daughter cell nearest the micropyle is usually delayed until after that in the daughter cell nearest the chalazal end. If this delay was carried a little farther, we should have the condition described in several other orchids (WARD '80, STRASBURGER '84), which have only three megaspores.

In Cypripedium (PACE '08) the daughter cells of the megaspore mother cell do not divide, but one of them forms the embryo sac. In this case the question arises as to whether the division of both daughter cells has been omitted, in the way indicated for one of them in Habenaria, and the place of the second "reducing division" changed to the embryo sac mother cell (the cell within the walls of which the embryo sac is organized); or whether the first two nuclei of the embryo sac are, as Miss PACE calls them, megaspore nuclei. In favor of the latter view it may be said that the "completion of chromosome reduction" which takes place in the division of the daughter cell is necessary to the normal development of the embryo sac. COULTER ('08) thinks that because chromosome reduction, which is usually associated with megaspore formation, is necessary that megaspore nuclei cannot be omitted. On the other hand, the place of reduction is not always constant, even in nearly related plants, as in the case of Nemalion (WOLFE '04) and Polysiphonia (YAMANOUCHI '05). In Nemalion the fertilized egg divides to carpospores and, according to WOLFE, reduction takes place in their formation. In Polysiphonia reduction does not take place in carpospore formation, but the carpospore germinates to a plant with the diploid number of chromosomes. This plant bears not carpospores but tetraspores, and reduction takes place in the division of the tetraspore mother cell. In speaking of Polysiphonia, YAMANOUCHI says: "The tetrasporic plants may have arisen by a suppression of the reduction phenomena in connection with the carpospore, so that it germinates with the sporophytic number of chromosomes. . . . . The period of chromosome reduction would be thus postponed from the carpospore to a later period in connection with the newly formed plant." If, as seems probable, the place of reduction has been changed among the red algae, it is reasonable to suppose that it may also have been changed in some angiosperms, and especially if the structure in which it normally occurred had been left out of the life-history of the plant. The leaving-out of the division of the the mother cell into megaspores would simply be the completion of a tendency toward the reduction in the number of divisions of the archesporial cells from the condition in the ferns (where it divides a number of times) to such a condition as Habenaria where the archesporial cell without dividing functions as a spore mother cell.

The only way in which we can claim that megaspore nuclei must accompany chromosome reduction is by defining a spore mother cell as that cell in which reduction is initiated and spore nuclei as the nuclei resulting from the reducing divisions. The logical conclusion of this would be to make chromosome behavior the sole criterion for distinguishing spores, sporophytes, and gametophytes; but since the four megaspores and embryo sac of Alchemilla (MURBECK '01) may have the diploid number of chromosomes, or the apogamous embryos of Nephrodium (YAMANOUCHI '08) the haploid number of chromosomes, we cannot regard chromosome behavior as the sole, if indeed the most important, criterion for distinguishing any of these structures.

A distinction between the first division of the megaspore and a division giving rise to megaspores is that while in the first case no cell plate is formed on the spindle, in the latter case either a wall or a cell plate is formed on the spindle. This wall may be formed in the embryo sac when this is derived from more than one megaspore, as is apparently the case in Peperomia (BROWN '08). The first division of the embryo sac mother cell nucleus of Cypripedium (PACE '08) agrees with that of those derived from one megaspore in having no cell plate formed on the spindle.

The evidence for either view is inconclusive, but seems to the writer to be in favor of the idea that in Cypripedium the second division in megaspore formation has been left out, and the place where "reduction is completed" changed to the first division of the nucleus of the embryo sac mother cell.

LLOYD ('02) and COULTER ('08) have advanced the idea that when megaspores are not formed the first four nuclei of the embryo sac are megaspore nuclei. This is probably true in such cases as Lilium (COULTER and CHAMBERLAIN '03) and Peperomia (BROWN '08), and as COULTER ('08) suggests in some other sixteen-nucleate embryo sacs; but if the ideas brought forward here concerning Cypripedium are correct, it need not be of universal application. CAMPBELL ('09), however, seems to think that even the embryo sacs of Lilium and Peperomia are not derived from more than one megaspore.

In discussing the view of COULTER ('08) that when the megaspore mother cell does not divide the first four nuclei of the embryo sac are megaspore nuclei, and of BROWN ('08) that the embryo sac of Peperomia is composed of the descendants of four megaspores, CAMPBELL ('09) says: "The generally accepted view that in such cases as Peperomia and Lilium the embryo sac is a single megaspore formed without previous division from the mother cell can hardly be admitted to have been disproved by these recent speculations." In speaking of Peperomia, he says that BROWN bases his opinion that the first four nuclei of the embryo sac represent megaspores upon the fact that cell walls are formed in the first two nuclear divisions in *Peperomia sintensii* and cell plates in the same divisions in *P. pellucida;* while in the third division cell plates are wanting. CAMPBELL says that since in the last division, which gives rise to sixteen nuclei, cell walls are formed, "this seems rather inadequate grounds for assuming that the embryo sac represents four spores rather than a single one." The presence of the cell walls was not the only reason for thinking that the embryo sac of Peperomia represents four spores; but even if it were, the formation of walls in the last division would offer no difficulty to such a view, for walls are usually formed at the last division of the embryo sac of angiosperms and the writer has not been able to find any essential difference between the method of their formation in Peperomia and in Habenaria. Nor would the reduction of the free divisions in the embryo sac to a single one (the third in Peperomia) be a difficulty when we remember that the number of these is often large but quite variable in the gymnosperms and has been reduced to two in the ordinary angiosperms. The fact remains that unless we assume that the first four nuclei of the embryo sac of Peperomia and Lilium are megaspore nuclei, we have no explanation for the presence of walls in the first two divisions of the embryo sac of Peperomia and for the absence of these walls in the third division, or for the presence of cell plates on the spindles of the first two divisions of Lilium, features which have been described in no case, so far as the writer knows, in which the embryo sac is formed from one of four megaspores.

CAMPBELL thinks that if the compound nature of the sac of Lilium be admitted, we still have to explain the extraordinary departure of Peperomia from the usual type, but why two such distantly related plants would be expected to behave alike is not explained.

It does not seem likely that a primitive embryo sac, as CAMPBELL believes the sixteen-nucleate type to be, has been retained in plants so far examined only in such distantly related genera as Peperomia, Pandanus (CAMPBELL '09), Gunnera (ERNST '08), Euphorbia (MODILEWSKI '09), and the Penaeaceae (STEPHENS '08); and especially since most of these genera are anything but primitive in other respects, and the embryo sac is in most cases derived from a spore mother cell which, as CAMPBELL says, can hardly be regarded as a primitive feature.

ENDOSPERM

NAWASCHIN ('00) finds that in Phajus and Arundina there is no fusion of the polar or second male nuclei, and he attributes the lack of endosperm to this cause. STRASBURGER ('00) has shown that in several European orchids this fusion may or may not take place, but in either case there is no division to form endosperm. He concludes from this that the lack of endosperm is not due to the failure of the nuclei to fuse. The condition in Habenaria, where there is no endosperm, although the fusion nucleus is of constant occurrence, is a confirmation of this view.

In contrast to Habenaria, endosperm may be formed in Lemna (CALDWELL '99) without a fusion of the polar nuclei.

In the aposporous embryo sac of Hieracium (ROSENBERG '06) polar nuclei with the diploid number of chromosomes may fuse to form the endosperm nucleus. In all known sixteen-nucleate sacs all of the nuclei not cut off by walls fuse to form the primary endosperm nucleus. In *Peperomia hispidula* (JOHNSON '07) there are fourteen of these fusing nuclei; while in *P. pellucida* (JOHNSON '00) and *P. sintensii* (BROWN '08) there are eight; in the Penaeaceae (STEPHENS '08) and *Euphorbia procera* (MODILEWSKI '09) there are four; and in Gunnera (ERNST '09) seven.

The fact that in Habenaria the fusion of the polar and second male nuclei does not result in the formation of endosperm, while in Lemna endosperm is formed without this fusion, taken together with the fact that the primary endosperm nucleus may be formed by the fusion of a variable number of nuclei or of nuclei with either the diploid or haploid number of chromosomes, seems to strengthen STRASBURGER'S ('05) view that the endosperm is not a sexually produced embryo, but that the fusion of the nuclei is connected with the fact that the nuclei have ceased developing and are in the same cell cavity.

Summary

The archesporium of Habenaria arises as a single hypodermal cell, which without dividing functions as a megaspore mother cell.

The mother cell divides to two daughter cells and each of these to two megaspores. The division of the daughter cell nearest the micropyle is usually delayed.

In some cases an embryo sac is probably formed from more than one megaspore; but the condition in the orchids, where there is a gradual reduction of the divisions of the megaspore mother cell without an indication of walls in the embryo sac, indicates that megaspore formation may be omitted and the place of reduction changed to the first division of the nucleus of the embryo sac mother cell.

The embryo sac of Habenaria contains eight nuclei: an egg, two synergids, two polar nuclei, and three ephemeral antipodals.

The primary endosperm nucleus is formed by the fusion of the polar and second male nuclei, but degenerates without dividing.

The absence of endosperm in many orchids offers no support to the view that the endosperm is a sexually produced embryo.

The fertilized egg gives rise to a long suspensor and a globular embryo.

My thanks are due to Professor D. S. JOHNSON for helpful suggestions and criticisms.

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